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in Persons with Different Habits of Exercise

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12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited			12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 Words) Evidence exists that women exercising have lower estrogen levels than sedentary women. These lower estrogen levels may be the mechanism behind their reduced breast cancer risk. Previous studies included athletes with high exercise levels, and estrogen measurements were based on a few serum samples from different times during a menstrual cycle. This study includes identical female twins who are discordant for moderate exercise. Estradiol is measured on a daily basis from saliva samples collected during a complete menstrual cycle. Procedures and questionnaires have been developed; enrollment of eligible pairs and laboratory assays are ongoing. Screening interviews have been conducted with 299 pairs. Of these, 56 were initially eligible; however 10 declined to participate and 2 later became ineligible due to menopausal related reasons. Samples proved to be unusable for 3 pairs. Thus, sample collection is currently completed or underway for 39 pairs. Estradiol and progesterone assays have been completed for 29 pairs. Preliminary results, based on these pairs indicated that E2 during luteal phase was significantly higher in the inactive member of the twin pair, while follicular phase E2 was unchanged. During the no-cost extension, we will finish sample collection from about 5 more pairs of twins, complete hormonal assays including daily progesterone, incorporate dietary data in the analysis and control for confounding factors using multivariate analysis. Several publications are being planned.			
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Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	5
Key Research Accomplishments.....	8
Reportable Outcomes.....	8
Conclusions.....	9
References.....	10
Appendices.....	14

4) INTRODUCTION

The purposes of the study are the following:

- (1) To determine the effect of moderate exercise on E2 levels during the follicular and luteal phases of (ovular) menstrual cycles by means of daily salivary samples in healthy premenopausal identical twins who differ in their amount of physical exercise activity per week.
- (2) To determine the effect of moderate exercise on frequency of anovulation and on menstrual cycle length (specifically luteal phase length) in identical twins who differ in their amount of physical exercise activity per week.

Overview: Exercise has been shown to be associated with a reduced risk of breast cancer [4,5,8,10,11]. There is evidence that women exercising, for an hour or more per day, have lower serum estrogen (estradiol) levels than sedentary women (due to more anovular cycles and lower estrogen levels in ovular cycles). These lower estrogen levels appear most likely to be the mechanism behind their reduced breast cancer risk, however much is still unknown. Previous studies have, for the most part, focused on the effects of high exercise levels among athletes, as opposed to more moderate levels of exercise, on estrogen levels, and they may have been subject to 'selection bias', i.e. women who exercise may do so because of predisposing hormonal factors. In addition, the estradiol measurements have usually been based on only a few serum samples taken at different times during a menstrual cycle. This study is addressing these issues by using 60 sets of monozygous twins who are discordant with regard to moderate exercise habits (i.e. sedentary vs. exercising an average of 20 minutes/day), but are identical for heritable aspects of body build and constitution. Estradiol is being measured on a daily basis by use of salivary samples collected during a complete menstrual cycle. The subjects are being selected from pairs of healthy premenopausal identical twins under the age of 45 who participated in the California Twin Cohort Study. They are being screened to determine eligibility (i.e. neither twin having an endocrine or metabolic disorder and the pair discordant for current amount of physical exercise activity), before being asked to participate. The use of the salivary samples is an innovative method for the measurement of estradiol and offers distinct advantages over the more traditional serum hormone measurements for which daily samples are not practical. Repeated sampling, as compared to single or infrequent sampling of individuals makes it possible to more accurately characterize ovarian function and allows for a more complete assessment of estradiol levels over different phases of the menstrual cycle, without the discomfort of venipuncture or the inconvenience of office visits. Salivary steroids have been shown to be extremely stable when samples are properly treated and this method of collection is ideally suited for use in the proposed study where subjects are located throughout California[43-46]. The hormone assays are being done by Dr. Peter Ellison (Co-Investigator), an expert in the analysis of and validation of salivary samples. We are also obtaining information on daily physical exercise activity during the month of sample collection and dietary intake using established and well tested questionnaires. Analysis of covariance methods will be used to assess the relationship of estrogen levels during different parts of the menstrual cycle to exercise, controlling for diet, body

mass, and other potentially confounding factors. Based on the sample size of 60 pairs of twins, we have the power to detect differences in estradiol levels of 15% between the sedentary and moderately exercising twins. The study has important public health implications in developing strategies for the prevention of breast cancer.

5) BODY

Technical Objectives and Work Accomplished:

Technical objectives 1-4: Selection of twins and collection of saliva samples: Ongoing throughout Yrs. 1, 2, and 3 during the no cost extension.

1. During the course of the study identical female twins will be selected who previously participated in the California Twin Cohort and indicated that they are premenopausal.
2. These pairs will be called on the telephone and re-interviewed regarding factors related to their eligibility.
3. Once a pair is determined to be eligible and they agree to participate they will be mailed informed consent forms, saliva sample collection kits, and exercise and dietary questionnaires.
4. We will check with them periodically to determine when the first day of their period occurs and assure that they are following the directions for collection of the saliva samples.
5. They will mail their completed sample kits to Dr. Ellison's laboratory and the completed questionnaires to USC.

Work accomplished on these objectives:

We originally selected 182 identical exercise discordant female pairs from the California Twin Cohort who were born before 1957 and were part of the first group of twins sent questionnaires in 1991-1992. During 1998 questionnaires were sent to additional California Cohort twins born before 1965 and 79 pairs have been selected from this group where both members of the pair participated. During 1999-2000 questionnaires were sent to California Cohort twin born before 1973 and 43 pairs were selected for screening. Of the 304 pairs, screening interviews have been conducted with 299 (Table 1), leaving 5 pairs still in process. During the past year we located 13 pairs who were previously considered to be lost by implementing tracing measures and sending letters to the last known address. From the 299 pairs, 56 (18.7%) were initially identified as eligible for the study; however 10 declined to participate (4 of these after receiving the kits) and 2 others later became ineligible due to menopausal related reasons (i.e. started taking HRT's). In addition, for five participating pairs, there were too many missing days of sample collection or other problems that caused their samples to be unuseable. Thus there are currently 39 pairs (13.0%) who have either completed the saliva collection process (29), or are in process (10) (Table 1).

Of those determined to be ineligible, the most common reasons were use of OC's or hormones (77) followed by parity discordance (i.e. one twin parous, the other nulliparous) (31), one both twins currently or recently pregnant or breast feeding (23), and one or both twins menopausal (22).

Due to higher rates of ineligibility than anticipated we have had fewer pairs participate than expected. We have contacted more pairs that originally planned in an effort to increase the sample size as much as possible. With these efforts we expect to achieve a sample size of approximately 40-45 completed pairs.

Table 1: Results of Screening Interviews

Result of Screening of Both Members of Pair	Number of Pairs	Percent of Total Pairs Screened
Eligible initially	56	18.7
And successfully participating	(39)	(13.0)
Participated, but not useable	(5)	(1.7)
And declined participation		
Before sending kits	(6)	(2.0)
After sending kits	(4)	(1.3)
And later became ineligible	(2)	(0.7)
Screened and not eligible because:	203	67.9
1+ had menopause	(22)	(7.4)
1+ had very irregular periods	(3)	(1.0)
Parity discordant	(31)	(10.4)
1 + had disqualifying disease	(8)	(2.7)
1+ taking OC's or hormones	(77)	(25.8)
1+ taking cortisone/prednisone	(9)	(3.0)
1+ breast fed a child or pregnant within past year	(23)	(7.7)
Multiple of above reasons	(11)	(3.7)
Both had same exercise level	(18)	(6.0)
One twin was deceased	(1)	(0.3)
Lost, could not screen	27	9.0
Refused screening interview	13	4.4
Total	299	100.0

Technical Objective 5: Completion of Hormonal Assays: Year 1, month 3 through Year 3, Month 9.

1. Dr. Ellison's Laboratory will receive the kits and will be blinded as to which twin is performing more exercise.
2. The laboratory assistant will complete the hormonal assays according to standard protocols.
3. Results will be sent to USC.

Dr. Ellison's Laboratory has processed samples from 29 pairs to date. Assays have been completed for daily estradiol levels and the mean midluteal progesterone levels. One twin was determined to have had a non-ovulatory cycle from these results. Two additional pairs completed the collection and sent the samples to Harvard; however their samples were not usable due to too many skipped days during the month. Another twin sent in saliva samples that contained only 1/4-1/3 the volume requested. Despite trying a second time, this twin was not able to provide a usable sample. This pair refused to use the chewing gum provided to increase saliva volume.

We plan to do more detailed daily analysis of progesterone levels since we will have few samples than originally anticipated.

Technical Objectives 6-7: Data Management: Year 1, Month 6-Year 3 Month 10

1. Physical Activity questionnaires will be coded and entered at USC.
2. Dietary questionnaires will be sent to Dr. Willett for analysis, with results being sent to USC.
3. Hormonal assay data will be merged with the questionnaire data.

An Access data entry program has been created for the questionnaire data and all of the received questionnaires have been entered. Data from the Willett dietary questionnaires from 46 individual twins have been received from Harvard. We have integrated the hormonal and physical activity questionnaire data for a preliminary analysis of the data.

Technical Objectives 8-9: Data analysis and publishing of papers: Year 1, Month 12-Year 3, Month 12. And Year 4 (no-cost extension).

1. Preliminary and final analyses will be performed to address the stated hypotheses.
2. Papers will be published on the results.

A poster was presented at the Era of Hope Meeting in Atlanta with preliminary results based on the first 15 pairs with laboratory and questionnaire data available. The results have been expanded to include data from 29 pairs and are summarized below.

Results: Hormone assays and questionnaires have been completed for 29 pairs. The midcycle day was determined by the day of the largest drop in E2. The average daily and total follicular phase and luteal phase E2 levels were calculated. Mid luteal phase progesterone levels were used to determine if the cycle was ovular. One twin was determined to have an anovular cycle and this pair was eliminated from the analysis. Both daily logs of physical activity and a general questionnaire completed prior to the sample collection were used to determine which twin was

more active. Preliminary data analysis based on 29 pairs indicates that average daily luteal phase estrogens were about 9% lower among the more active twins compared to the less active twins (21.9 vs. 24.1 pmol/L) and total luteal phase E2 was about 12% lower among the more active twins (271.9 vs 295.5 pmol/L) (Table 2). In contrast, there was little difference in average daily or total follicular phase E2 by activity level (133.2 vs 134.6 pmol/L). The difference of means for the average daily luteal phase estrogen was significant based on the paired T test. None of the other estrogen related variable differences was statistically significant. The more active twin averaged slightly more hours of sports and recreational activity than the less active twin (3.8 vs. 3.3 hours) and had a significantly higher proportion of weekday time in vigorous or moderate activity was significantly more likely to participate in a regular exercise program at least at least once a week (76% vs. 50%). Among other variables compared the body mass index of the more active twin was significantly lower than that of the less active twin (23.3 vs. 24.5).

6) Key Research Accomplishments

- We have demonstrated that the large majority of women participating are able to collect the saliva samples as requested and mail them to the Laboratory for analysis.
- We have developed the study materials and protocols
- Preliminary analyses have indicated that average daily luteal phase estrogen levels were lower in the more active twin, whereas no difference was found in follicular phase estrogens.

7) Reportable Outcomes

Hamilton, Ann, Lipson, Susan, Shames, Lisa, and Ellison, Peter , The Effect of Physical Activity on Menstrual Cycle Estrogens: A Study of Identical Twins, poster presented at the Era of Hope Meeting, Atlanta, Georgia, June, 2000.

Due to the successful collaboration in this study with Dr. Ellison, a second grant involving use of saliva assays has been submitted (to the state of California) to study testosterone levels in males at risk of testis cancer.

A student interested in exercise physiology received training in research on this grant as she contacted the twins and conducted the screening interviews. At Dr. Ellison's Laboratory, a graduate student participated in the estradiol assays. A graduate student in biostatistics has assisted Dr. Hamilton in developing an index to determine the more active twin based on the main questionnaire and on the daily logs. Reliability between the two methods has been assessed.

Several publications are being planned including:

- 1) Non-invasive methods of obtaining estrogen levels from a complete menstrual cycle
- 2) Reliability of multiple assessments of physical activity levels
- 3) Effect of moderate physical activity on menstrual cycle estrogens

Table 2: Estrogen and Activity* Related Variables by Activity Status of Twin: 29 Identical Female Twin Pairs

	More Active Twin	Less Active Twin
Estrogen Related Variables		
Average Luteal Phase E2/Day***	21.9 (pmol/L)	24.1 (pmol/L)
Average Luteal Length ^a	12.6 days	12.1 days
Total Luteal E2	271.9 (pmol/L)	295.5 (pmol/L)
Average Follicular Phase E2/Day	22.3 (pmol/L)	22.5 (pmol/L)
Average Follicular Length ^b	5.7 days	6.1 days
Total Follicular E2	133.2 (pmol/L)	134.6 (pmol/L)
Activity Related Variables		
Hours of physical activity/week	3.8 hours	3.3 hours
Hours of physical activity/life**	1192.7 hours	1321.8 hours
Flights of stairs/day	6.7	6.6
Blocks walked/day	6.6	5.3
Proportion of ave. weekday in vigorous or moderate activity***	21%	15%
Proportion of ave. weekend day in vigorous or moderate activity	24%	21%
Proportion getting regular exercise at least once/week***	76%	50%
Other variables		
Body Mass Index (kg/m ²)***	23.3	24.5
Age at menarche	12.7 years	12.6 years
Age at first pregnancy	22.6 years	22.0 years
Number of pregnancies	2.9	2.9

*Activity status based on higher index of activity on variables listed below under 'Activity-related variables' based on daily logs (or on main questionnaire, if daily logs not completed).

^a Luteal phase defined as 1 day after midcycle day until onset of next period.

^b Follicular phase defined as 4 days after onset of period until 4 days before midcycle day.

**Activity over life time is based only on those activities that were participated in during the past year.

***p=<.05 for difference between more active and less active twin.

8) Conclusions

There is a suggestion that lower average daily luteal phase estrogens may be related to higher activity level in these identical pairs. Since more exposure to E2 occurs during the luteal phase, the reduction in exposure due to exercise may be significant over many years. The findings have implications for the influence of moderate exercise on ovarian function in non-athletes. More thorough understanding of the role of moderate exercise has important public health consequences and will be useful in determining strategies for reducing breast cancer risk.

We will expand these results to the complete data set, include the information on diet, and conduct multivariate analyses for the final analysis. Progesterone levels will also be compared.

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10) APPENDICES

1. IRB Approval and Informed Consent

INSTITUTIONAL REVIEW BOARD
HEALTH RESEARCH ASSOCIATION
AND
INSTITUTIONAL REVIEW BOARD
UNIVERSITY OF SOUTHERN CALIFORNIA
SCHOOL OF MEDICINE

Date: 09/24/01
To: **Ann S Hamilton, Ph.D.**
Assistant Professor
Preventive Medicine
Norris Cancer Center, #3427
HEALTH SCIENCES CAMPUS

From: **Vice Chair, IRB**
Robert Larsen, M.D.
Trailer #25, Unit I
1200 N. State Street
Los Angeles, CA 90033-

(323) 223-2340/2349

TITLE OF PROPOSAL:
**AN INNOVATIVE ASSESSMENT OF ENDOGENOUS ESTROGEN ACTIVITY IN PERSONS WITH
DIFFERENT HABITS OF EXERCISE**

Action Date: 9/14/01 Action Taken: Approved

Committee: Vice Chair, IRB

Note:

Your correspondence dated 8/21/01 and attachments were reviewed and APPROVED by Dr. Robert Larsen on 9/14/01.

Based on expedited review of your response, IRB stipulations from 8/16/01 have been fully satisfied.

The informed consent form dated 8/4/00 was approved on this date.

**APPROVAL FOR YOUR STUDY WILL EXPIRE AT THE END OF THE DAY (i.e.,
MIDNIGHT) ON 8/15/02.** IRB approval is valid for a maximum period of one year with continuing review by the IRB required at least annually in order to maintain approval status. You may not enter subjects on the study before IRB approval or if IRB approval expires. In the latter case you must immediately contact the IRB to obtain permission to continue subjects on the trial. You must submit a progress report (Continuing Review Form) sufficiently (one to two months) prior to your study expiration date to permit IRB review before the expiration date. You will receive a new informed consent form to use for the following year, if your project is approved. raf/RAL

Initials: _____

USC Norris Comprehensive Cancer Center
Keck School of Medicine
Department of Preventive Medicine
1441 Eastlake Ave., Rm. 3427, MC9175
Los Angeles, CA 90089-9175



**Description of the Medical Research for which your participation is requested
(Please sign and return one copy and keep the second copy for your records)**

TITLE OF PROJECT: An Innovative Assessment of Endogenous Estrogen Activity in Persons with Different Habits of Exercise

PRINCIPAL INVESTIGATOR: Ann Hamilton, Ph.D. (323)-865-0434

DEPARTMENT: Department of Preventive Medicine, Keck School of Medicine at the University of Southern California, 1441 Eastlake Ave, MC9175, Los Angeles, CA 90089.

24-HOUR TELEPHONE NUMBER: Toll free number: 800-421-9631

PURPOSE OF THE STUDY: You are invited to participate in a research study that is being conducted to learn more about the effects of physical activity on ovarian hormones in pre-menopausal women. You are invited to be a participant because you are a member of an identical twin pair.

PROCEDURES: Your participation in this study involves three aspects. First, you will be asked to complete a survey regarding your exercise habits, dietary intake and personal medical history. Second, you will be asked to collect 5ml's (approximately 5 teaspoons) of saliva (in a plastic tube) over an entire menstrual cycle (or approximately 30 days) by spitting into a tube. The saliva collection should be done one time per day when you first wake up in the morning. Detailed information on how the saliva samples are to be collected are included in the study packet on a form entitled 'Instruction for Participants'. The tube is pre-treated with a small amount of a bactericidal agent called sodium azide. The sodium azide is at the bottom of each tube and is visible as a small dried residue which adheres firmly to the base of each tube. Please keep tubes out of the reach of small children since this agent is toxic when ingested. Lastly, you will be asked to keep a daily log of your physical activities (we will supply you with these forms) over one menstrual cycle.

The entire study will be performed over one menstrual cycle (or about 30 days) and will be done in the convenience of your home. We will mail you a study packet complete with instructions, collection tubes, and surveys. The packet should be mailed to the laboratory upon completion of your participation in the study (we will pre-pay postage).

RISKS: There are no conceivable health risks associated with the collection of one's own saliva. However, care should be exercised when storing tubes in a home with small children, since the bactericidal agent adhering to the bottom of each tube (which keeps the specimens fresh), can be toxic if swallowed.

Initials: _____

BENEFITS: There are no direct benefits to you. There is, however, the indirect benefit of knowing that you are contributing to a study that may help us better understand how physical activity affects women's health.

ALTERNATIVES TO PARTICIPATION: It is understood that you may choose not to participate in this study and that your decision will not in any manner affect your medical care. This study is designed to assess hormone levels over an entire menstrual cycle and there are no other convenient non-invasive alternatives to this method of sample collection. You are free to withdraw your consent and discontinue participation at any time.

CONFIDENTIALITY: Every effort will be made to maintain the confidentiality of your medical records for this study by the investigator and the Institutional Review Board (IRB) to the extent permitted by law. Any information which personally identifies you will not be released or disclosed without your written consent except as specifically required by law. Representatives of the U.S. Army Medical Research and Materiel Command are eligible to review research records as part of their responsibility to protect human subjects in research.

OFFER TO ANSWER QUESTIONS: It is understood that if you have any questions, comments or concerns about the study or the informed consent process, you should contact the Principal Investigator; Dr. Ann Hamilton with the Department of Preventive Medicine, 1441 Eastlake Ave. MC9175, Los Angeles, CA 90089-9175 ((323) 865-0434). If you have any questions regarding your rights as a study participant, you may contact the Institutional Review Board Office at (323) 223-2340. You will be given a copy of this form to keep.

COMPENSATION: The availability and quality of your medical care will not be affected by your participation or refusal to participate. We can provide no compensation for your participation in this study.

VOLUNTARY PARTICIPATION AND WITHDRAWAL: Your participation in this research study is voluntary. Your decision whether or not to participate will not interfere with your right to health care or other services to which you are otherwise entitled. If you do decide to participate, you are free to withdraw your consent and discontinue participation at any time.

INJURY STATEMENT: If you should require medical treatment as a result of an injury arising from your participation in this study, the financial responsibility for such treatment will be yours.

NEW INFORMATION: Any new information that is developed during the course of this research which may be related to your willingness to continue or discontinue participation in this study will be provided to you.

POTENTIAL FOR COMMERCIAL DEVELOPMENT RELATED TO RESEARCH AND DONATION OF SALIVA SAMPLES. The following paragraph is included at the request of the funding agency for the study, the Department of Defense. We feel that the possibility of a commercial application is remote.

There is the possibility that the saliva samples provided may also be used in other research studies and could potentially have some commercial applicability. You are being asked to

voluntarily and freely donate the saliva samples to the Keck School of Medicine at the University of Southern California and relinquish all right, title, and interest to said items.

I voluntarily and freely donate any and all saliva samples to the Keck School of Medicine and hereby relinquish all right, title, and interest to said items.

Please write your initials in the box to indicate your agreement. Thank you.

CONSENT PROCEDURE: Initial contact with participants in this study is made by telephone to determine if the participant is eligible for the study. Then a letter describing the study purpose and procedures is sent. Participants are asked to sign and return a copy of this document.

CALIFORNIA LAW REQUIRES THAT YOU MUST BE INFORMED ABOUT:

1. The nature and purpose of the study.
2. The procedures in the study and any drug or device to be used.
3. Discomforts and risks to be expected from the study.
4. Benefits to be expected from the study.
5. Alternative procedures, drugs or devices that might be helpful and their risks and benefits.
6. Availability of medical treatment should complications occur.
7. The opportunity to ask questions about the study or the procedure.
8. The opportunity to withdraw at any time without affecting your future care at this institution.
9. A copy of the written consent form for the study.
10. The opportunity to consent freely to the study without the use of coercion.
11. Statement regarding liability for research-related injury, if applicable.

AGREEMENT:

I have read (or someone has read to me) the information provided above. I have been given the opportunity to ask questions and all of my questions have been answered to my satisfaction. My signature below indicates that I have decided to participate having read the information provided above.

Name of Subject	Signature	Date Signed
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Thank you! Please mail the signed copy in the enclosed postage paid envelope or to the address listed below.

Dr. Ann Hamilton
USC/Norris Comprehensive Cancer Center
1441 Eastlake Ave., Rm 3427, MC9175
Los Angeles, CA 90089-9175

Form Valid For Enrollment From		
SEP 14 2001	To	AUG 15 2002
Institutional Review Board		